DNA-Mediated Gene Therapy in a Mouse Model of Limb Girdle Muscular Dystrophy 2B.

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Authors: Julia Ma, Christophe Pichavant, Haley du Bois, Mital Bhakta, Michele P Calos

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Public Summary:
This paper demonstrated an effective method to correct the mutated gene and a form of limb girdle muscular dystrophy. A plasmid encoding the therapeutic gene, dysferlin, with or without the follistatin gene, was introduced into hind limb muscles of a mouse model of the disease. A vascular injection method was used to distributed the therapeutic DNA to all the major hind limb muscles. The follistatin gene was included in some experiments, because this gene product has been shown to stimulate beneficial anabolic changes in muscles. The role of follistatin may include stimulation of the proliferation of muscle stem cells, leading to the formation of new muscle fibers, as well as growth of existing muscle fibers. The study demonstrated that the delivery method was successful in producing abundant quantities of the therapeutic protein in the muscles that persisted long-term. Muscles were examined three months after the therapy by the Evans blue assay, which measures the permeability of the muscles to an injected dye. Elevated permeability is a feature of the disease, and we were able to show that our therapy reduced this pathological permeability. This result suggested that the treatment was effective in improving the muscle health in this form of muscular dystrophy. We will build on these findings to develop therapies that could progress to the clinic.

Scientific Abstract:
Mutations in the gene for dysferlin cause a degenerative disorder of skeletal muscle known as limb girdle muscular dystrophy 2B. To achieve gene delivery of plasmids encoding dysferlin to hind limb muscles of dysferlin knockout mice, we used a vascular injection method that perfused naked plasmid DNA into all major muscle groups of the hind limb. We monitored delivery by luciferase live imaging and western blot, confirming strong dysferlin expression that persisted over the 3-month time course of the experiment. Co-delivery of the follistatin gene, which may promote muscle growth, was monitored by ELISA. Immunohistochemistry documented the presence of dysferlin in muscle fibers in treated limbs, and PCR confirmed the presence of plasmid DNA. Because dysferlin is involved in repair of the sarcolemmal membrane, dysferlin loss leads to fragile sarcolemmal membranes that can be detected by permeability to Evan's blue dye. We showed that after gene therapy with a plasmid encoding both dysferlin and follistatin, statistically significant reduction in Evan's blue dye permeability was present in hamstring muscles. These results suggest that vascular delivery of plasmids carrying these therapeutic genes may lead to simple and effective approaches for improving the clinical condition of limb girdle muscular dystrophy 2B.

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